Measurement of Crystallinity and Phase Composition of Hydroxyapatite by XRD

VAMAS TWA#3 Project #14

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BACKGROUND and APPROACH

Hydroxyapatite (HA) is a ceramic material increasingly used as a biocompatible monolithic material or a coating encouraging adaptation of an implant into the human body, but the body's response depends critically on the phase composition of the material. In particular as small changes in Ca:P ratio (and hence phase composition) have been demonstrated to have a profound effect on the biological response they elicit in vivo, it is important that standard methods of analysis and reporting are employed to ensure that all data are comparable. The objective of this round robin was to assess the level of accuracy and repeatability achievable by a new method proposed as an ISO standard for the quantification of HA crystallinity and phase composition. This new method differs from the usually cited method and potentially removes uncertainties stemming from overlapping peaks.

The XRD pattern for HA has many peaks in the range from about 7° to about 60° 2θ CuKα. The most intense peaks are in the range 30 to 35°, which coincides with the peak of the amorphous band if amorphous calcium phosphates are also present. Impurity phases such as α- and β-tricalcium phosphate (TCP), even CaO, can also be present and must be controlled at low levels for appropriate bioactivity of the product. To measure these at low levels in HA means that the peaks must be identified, separated and background stripped. Figure 1 illustrates the problem. The minor impurity phase is very close to the main HA peaks, and is difficult to separate effectively. In addition, there can be an amorphous background from uncrystallized or poorly crystallized HA as illustrated in Figure 2.
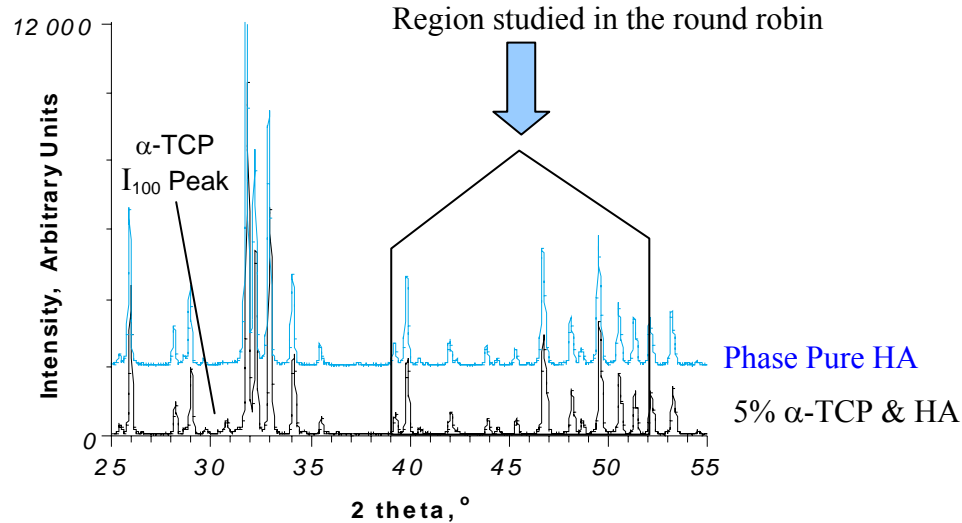
Figure 1. XRD patterns obtained from phase pure HA and α-TCP phase impure HA.
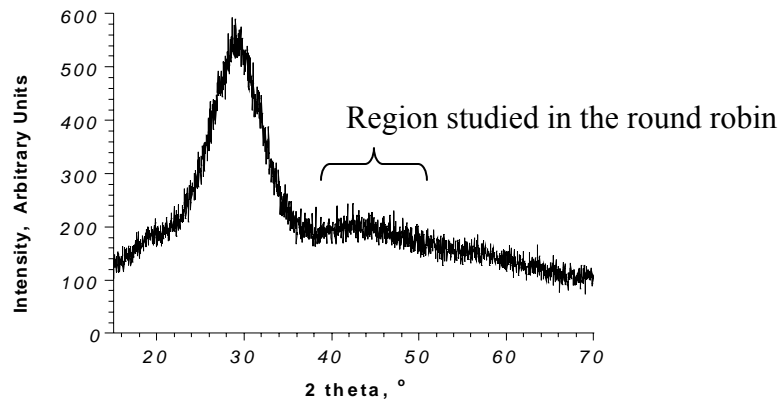
Figure 2. XRD trace of a calcium phosphate glass simulating amorphous HA.

The main peak is centered under the principal lines of HA and of the impurities. If these peaks are to be used for the estimation of phase content, this background has to be both estimated and removed, which is difficult to do in a reliable fashion.

The method proposed to ISO, and tested out in the round robin, is to use a different part of the XRD pattern to perform the calculations, specifically the 39°-52° 2θ portion (Figure 1). In this region, amorphous calcium phosphate displays a second band of amorphous scatter (Figure 2, arrowed region), but of much lower intensity. There are no significant impurity peaks present in this band. Given that impurities are typically present as less than 5% of the total crystalline phases present in the sample, choosing this region significantly reduces the potential for inaccuracies due to overlapping of peaks characteristic of different phases, and removes the necessity to deconvolute the pattern.

Participants in the round robin received quantities of pure HA, a calcium phosphate glass, α- and β-TCP and CaO as the reference powders for calibrations. A total of 10 combinations of these materials were mixed up and the X-ray patterns determined to provide the calibrations needed. Participants then prepared XRD traces of the sample powders in a consistent manner over the range of at least 20° to 55° 2θ in order to provide the zones for analysis. Analysis of the calibration samples provided the calibration parameters which were then used to estimate the phase proportions in the unknowns. Data were returned using a spreadsheet system, together with experimental details, particularly of the software systems employed, and any experimental difficulties encountered.

In performing the data analysis it was clear that the method could be further improved (generally by simple changes to the calibration calculations), and to this effect, some of the participants suggested a number of changes to the method to improve precision of the analysis.

SUMMARY of RESULTS

- Mean levels of **reproducibility** for the method (interlab variation) when analysing the standard 'unknown' powder samples was found to be:
- ± 0.5% on second phases and ± 1.8% on the HA in high crystallinity samples.
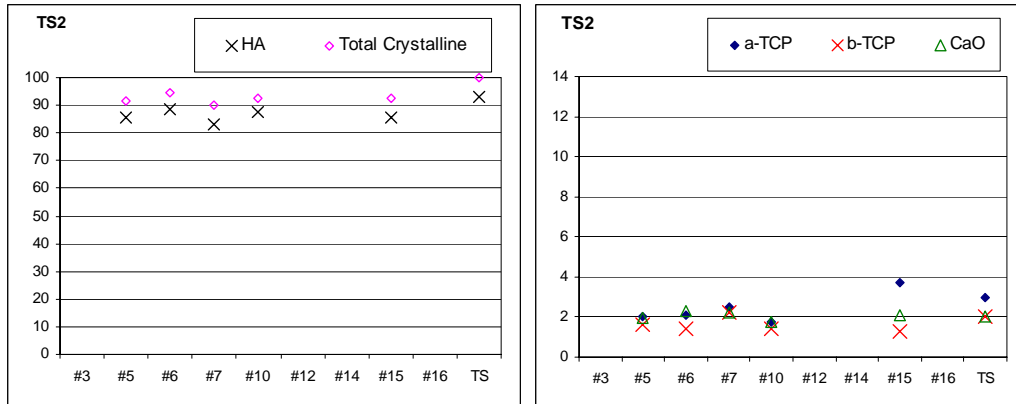- ± 1.1% on second phases and ± 4.0 % on the HA in low crystallinity samples.

Fig. 3. Scatter on analysis data of same *high* crystallinity 'unknown' from different labs. The horizontal axis represents the different labs. The last entry on the right, labelled TS for technical specification, denotes the specified content that was mixed up by each participant from the furnished reference powders. The vertical axes are the percent crystallinity. TS2 in the top left denote mix #2, or technical specification combination #2.
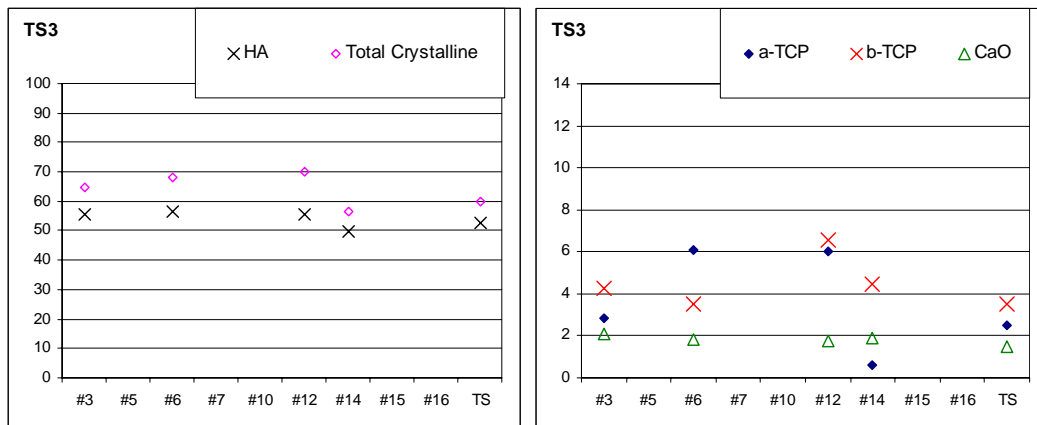

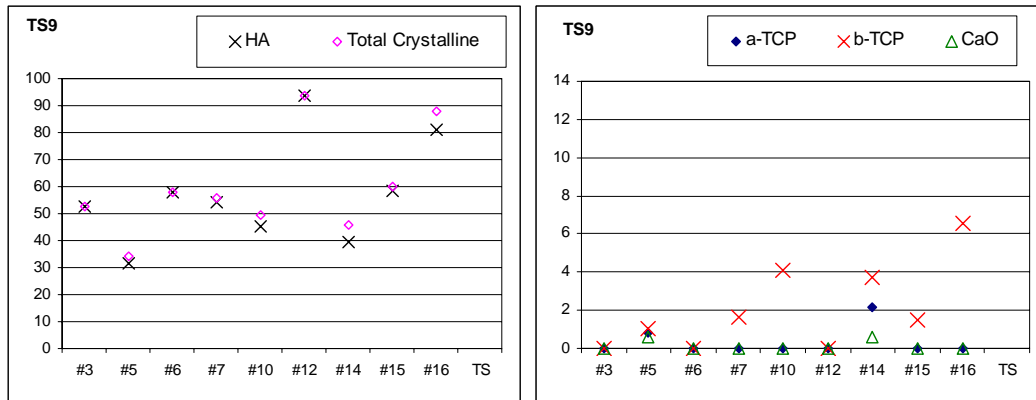Fig 4. Scatter on analysis data of same *low* crystallinity 'unknown' from different labs.

Fig 5. Mean levels of *reproducibility* for the method (interlab variation) when analysing the plasma sprayed 'unknown' sample was found to be significant.

Table 1.

The method underestimated the total crystalline content in highly crystalline materials, resulting in incorrect classification of 90% of samples containing >95% HA & 95% of samples with >95% crystalline content as having failed the specification.

Spec. of Test Sample		Results of Analysis	
% Crystalline phase	% Crystalline HA	% Crystalline phase	% Crystalline HA
100	93	90 ± 1	85 ± 1
100	93	92 ± 2	86 ± 2
100	95	92 ± 1	87 ± 2
100	97	93 ± 4	89 ± 4

Table 2.

Analysis of second phase impurities in highly crystalline materials, resulted in correct classification of 65% of samples as having passed/failed the specification of < 5% crystalline other phases.

Spec. of Test Sample			Results of Analysis		
% α-TCP	% β-TCP	% CaO	% α-TCP	% β-TCP	% CaO
0	0	5	0	0	4.8 ± 0.5
3	2	2	2.4 ± 0.8	1.6 ± 0.4	2.0 ± 0.2
3	0	0	3.4 ± 1.4	0.1 ± 0.2	0.1 ± 0.2
3	4	0	1.9 ± 0.9	2.7 ± 0.9	0.4 ± 0.5

Table 3.

The method proved more suited to measurement of 'less crystalline' specimens, but still incorrectly classified 21% of samples containing > 45% crystalline phases and 30% of samples containing > 45% as crystalline HA as having failed the specification.

Spec. of Test Sample		Results of Analysis	
% Crystalline phase	% Crystalline HA	% Crystalline phase	% Crystalline HA
47	43	45 ± 6	42 ± 7
52	47	53 ± 3	42 ± 2
60	53	65 ± 6	54 ± 3
60	50	63 ± 4	50 ± 4

Table 4.

Analysis of second phase impurities in less crystalline materials, resulted in correct classification of 71% of samples as having passed/failed the specification of < 5% crystalline other phases.

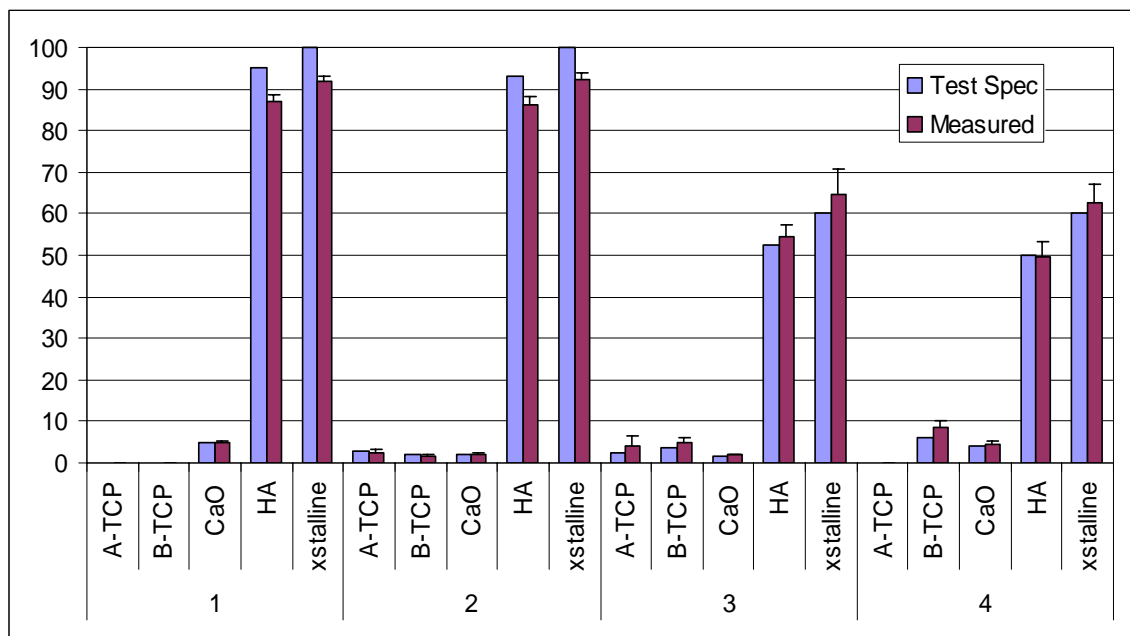| Spec. of Test Sample			Results of Analysis		
% α-TCP	% β-TCP	% CaO	% α-TCP	% β-TCP	% CaO
4	0	0	3.8 ± 1.9	0	0
3	2	0	5.5 ± 3.0	5.3 ± 1.9	0.2 ± 0.1
3	4	2	3.9 ± 2.7	4.7 ± 1.3	1.9 ± 0.1
0	6	4	0	8.5 ± 1.8	4.4 ± 0.7

Fig. 6. Graphical summary of analysis data on 4 of the 'unknowns.'

CONCLUSIONS

The method reproducibility for powder specimens is acceptable.

The method accuracy is unacceptable.

Should the accuracy be improved sufficiently by re-analysis of data using an exponential fit on the amorphous/crystalline calibration data it has been recommended that the ISO group adopt the method ensuring that its limitations are understood by users through inclusion of accuracy tolerances.